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ABSTRACT:

The present invention relates to a process for in vitro support of mammalian hematopoietic cells derived from peripheral blood. The hematopoietic cells may be stem cells and/or progenitor cells and the cells are maintained in a culture medium which contains at least one cytokine effective for supporting such cells. Additionally the present invention also relates to a process for treating a patient by cell replacement therapy by administering to the patient the hematopoietic cells derived from peripheral blood and maintained in vitro.

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(54) Title: PROCESS FOR SUPPORTING HEMATOPOIETIC PROGENITOR CELLS

(57) Abstract

The present invention relates to a process for *in vitro* support of mammalian hematopoietic cells derived from peripheral blood. The hematopoietic cells may be stem cells and/or progenitor cells and the cells are maintained in a culture medium which contains at least one cytokine effective for supporting such cells. Additionally the present invention also relates to a process for treating a patient by cell replacement therapy by administering to the patient the hematopoietic cells derived from peripheral blood and maintained *in vitro*.

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PROCESS FOR SUPPORTING HEMATOPOIETIC
PROGENITOR CELLS

The present invention provides a process for _in vitro_
support of mammalian hematopoietic cells derived from peripheral
blood.

BACKGROUND OF THE INVENTION

The extraordinary proliferative capacity of hematopoietic
progenitors derived from bone marrow _in vitro_ was initially
recognized through their ability to form large colonies under
semi-solid culture conditions (1,2). Following the development
of these techniques, the semi-solid culture method for
characterizing hematopoietic progenitors has been used as a
standard for evaluating in vitro culture techniques (3-6).

An alternative method for the culture of hematopoietic cells
in the murine system was developed by Dexter _et_ _al._ (7,8) which
relied upon the endogenous production of growth factors by an
adherent stromal cell layer which had been previously
established. With this technique, the cultures could be
maintained for many months. Co-culture of hematopoietic cells on
a stromal layer has subsequently been used to evaluate a number
of _in vitro_ culture properties (9-11). More recent studies have

concentrated on reconstituting the hematopoietic system of patients following chemotherapy using bone marrow mononuclear cells cultured in vitro without cytokines (19-20).

Methods for supporting bone marrow in the absence of a preestablished stromal layer have been developed (12-18, 30). These techniques involve culturing hematopoietic cells in static culture (T-flasks and multi-well plates) using traditional tissue culture techniques with added cytokines under a variety of conditions.

Effects of the _in vivo_ use of hematopoietic growth factors are currently being elucidated (21-23, 29). A key effect of a subset of these growth factors is an increase in committed progenitor cells in the peripheral blood. These cells can be harvested by leukophoresis for reinfusion following chemo/radiation therapy. Several studies have demonstrated the transplantation potential of hematopoietic cells retrieved from peripheral blood following _in vivo_ enrichment with G-CSF, GM-CSF, or IL-3 (24-28). None of these studies has expanded these hematopoietic cells _in vitro_ for use in transplantation.

Relevant patents include a process for replicating bone marrow _in vitro_ (US patent number 4,721,096 (1988)) and separation and maintenance of stem cells (US patent number 5,061,620 (1991)). To date, no study has demonstrated in vitro expansion of progenitors from peripheral blood for use in' transplantation.

SUMMARY OF THE INVENTION

The present invention provides for a process for _in vitro_ support of mammalian hematopoietic cells derived from peripheral blood. These cells can be used in addition to or in place of bone marrow cells for transplatation. Specifically, the cells are stem cells and/or progenitor cells and they are maintained in a culture medium which contains at least one cytokine effective for supporting such cells.

Additionally, the cells may be contained in a number of culture vessels such as a stir tank or airlift bioreactor (with or without the use of microcarrier beads), blood bags or growth chambers in which there is a continuous or periodic addition or perfusion of growth medium.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1

Results of the total cell number increase for culturing previously frozen bone marrow low density cells in spinner vessel bioreactors with and without microcarrier beads.

Figure 2

Results of the total cell number increase for culturing previously frozen bone marrow low density cells in an airlift bioreactor.

Figure 3

Total cell expansion for enriched low density peripheral blood mononuclear cells (PBMC) in spinner vessels. PBMC's were enriched by treating patients in vivo with G-CSF. Although both cultures were seeded at 5 x 10^5 cells/ml, the frozen culture may have been enriched for more immature cell types due to the freeze-thaw process. Plates A and B represent duplicate experiments.

Figure 4

Total cell expansion for enriched low density PBMC's in six well plates as determined by Trypan blue stained cell counts. Although both cultures were seeded at 5 X 10^5 cells/ml, the frozen culture may have been enriched for more immature cell types due to the freeze-thaw process.

Figure 5

Expansion of the granulocyte-macrophage progenitor cells (CFU-GM) from enriched low density PBMC's in spinner vessel bioreactors as determined by methylcellulose colony assays.

Figure 6

Expansion of erythroid progenitor cells (BFU-e) from enriched low density PBMC's in spinner vessel bioreactors as determined by methylcellulose colony assays.

Table I: Maximum expansion values for the total cells, CFU-GM, and BFU-e exhibited by previously frozen bone marrow, fresh PBMC's and frozen PBMC's in various small scale bioreactors. All expansion values were normalized to a value of 1.0 at the start of the experiments. Also listed is the timepoint at which the maximum expansion occurred. PBMC's were obtained from patients treated in vivo with G-CSF.

Detailed Description of the Invention

An object of the present invention provides for the in vitro support of mammalian hematopoietic cells derived from peripheral blood. The cells are maintained in a culture medium which contains at least one cytokine effective for supporting such cells.

Preferred embodiments of this aspect of the present invention provide a process for supporting mammalian hematopoietic cells derived from peripheral blood which are progenitor cells, and a process for supporting hematopoietic cells which are derived from peripheral blood which are stem cells.

Prior to Applicants' invention such hematopoietic cells had not been successfully supported in vitro. Additionally, Applicants' invention provides for the support of hematopoietic stem and progenitor cells derived from PBMC without prior selection of progenitor cell populations as used in expanding cells derived from bone marrow.

The term "stem cell" as used herein and by those skilled in the art means cells which are not committed and which give rise to more differentiated cells and other stem cells. The term "progenitor cells" as used herein and by those skilled in the art

means a committed or specialized precursor with proliferative capability. The term "supporting" as used herein and by those skilled in the art with respect to stem, progenitor and other hematopoietic cells means maintaining and/or expanding and/or promoting some differentiation of such cells.

The following are representative of cytokines effective for supporting the expansion of cells in accordance with the present invention:interleukin (IL)-1; IL-3; IL-6; granulocyte/ macrophage-colony stimulating factor (GM-CSF); human or murine stem cell factor (sometimes referred to as human or murine mast cell growth factor (MGF) or a c-kit ligand).

Another aspect of the present invention provides for a process of supporting hematopoietic cells wherein the cells are maintained in a culture medium containing a combination of cytokines effective for supporting such cells. The following are representative of some examples of such combinations of cytokines: IL-3 and GM-CSF; IL-3, GM-CSF and stem cell factor. The representative examples of cytokines and combinations thereof are merely intended to be examples of some cytokines and is not intended to limit the present invention.

In accordance with another aspect of the present invention, there is provided a process for supporting mammalian hematopoietic cells by maintaining the cells in a culture medium which contains at least one cytokine effective for supporting such cells, wherein the culture is maintained within a large scale cell culture system, for example, a stirred tank (spinner vessel) bioreactor or an airlift bioreactor. In a preferred embodiment of this aspect of the present invention, the bioreactor used is the stirred tank bioreactor which may be utilized with or without micro-carrier beads to aid in the expansion of the cells in the bioreactor. Such cells may be derived from peripheral blood or from bone marrow. The use of large reactors provides distinct advantages over conventional T-flask cultures in the expansion of progenitor and stem cells on

a large scale where the cells have been derived from either peripheral blood or bone marrow.

An advantage of a spinner vessel or airlift bioreactor is that the large number of cells necessary for transplantation can be cultured in one or a few vessels whereas culturing these cells in T-flasks would require many vessels. This significantly reduces the amount of labor required to perform these in vitro cultures and reduces the likelihood of contamination observed from these additional manipulations. In addition, improved expansion of the cells is obtained. Figures 1 and 2 are examples of the expansion of cells in spinner vessels and airlift bioractors.

The term "stirred tank (spinner vessel) bioreactor" as used herein and by those skilled in the art means any vessel used to contain cells utilizing an impeller and/or magnetic stir bar for medium agitation containing at least one port for the addition and removal of medium or other nutrients. The term "airlift bioreactor" as used herein and by those skilled in the art means any vessel used to contain cells using a sparged or bubbled gas for medium agitation and/or aeration containing at least one port for the addition and removal of medium or other nutrients. The term "microcarrier bead" as used herein and by those skilled in the art means any bead (porous or non-porous) used to support or enhance the proliferation of cells in agitated vessels.

The following are representative examples of cytokines which may be employed in the present invention: IL-1 may be employed in an amount effective to support the cells, generally, such amount is at least 20 pg/ml and need not exceed 1 ng/ml, preferably 1 ng/ml; IL-6 may be employed in in an amount effective to support the cells, generally, such amount is at least 1 pg/ml and need not exceed 50 ng/ml preferably 10 ng/ml; IL-3 may be employed in an amount effective to support the cells, generally, such amount is at least 500 pg/ml and need not exceed 50 ng/ml, preferably 500 pg/ml; GM-CSF may be employed in an

amount effective to support the cells, generally, such amount is
at least 100 pg/ml and need not exceed 1 ng/ml, preferably 200
pg/ml; c-kit ligand may be employed in an amount effective to
support the cells, generally, such amount is at least 1.0 ng/ml
and need not exceed 500 ng/ml, preferably 100 ng/ml. Such
cytokines may be employed alone or in combination with each
other.

The present invention provides that hematopoietic cells may
be derived from peripheral blood withdrawn from a mammal in
particular, a human. A preferred embodiment of the present
invention provides that the mammal has been treated with
cytokines to enrich progenitor cells prior to the withdrawal of
blood.

The cells supported in accordance with the present invention
may be used in a variety of ways. For example, such cells may be
employed as part of cell replacement therapy. Specifically the
expanded and cultivated cells may be infused alone or added to
bone marrow cells for bone marrow transplant procedures.

The expanded cells produced in accordance with the present
invention may be used in cell replacement therapy.

Such a procedure would involve treating the patient with one
or a combination of cytokines in particular G-CSF or by using
other agents which increase the number of stem cells or
progenitor cells in the peripheral blood. Peripheral blood
leukocytes are then harvested by leukapheresis three times at
intervals of three days before starting chemotherapy. Peripheral
blood mononuclear cells (PBMC) are obtained by density
centrifugation. A typical leukapheresis results in approximately
1.4×10^5 CFU-GM progenitor cells per kilogram of patient. The
total PBMC harvest for the process is typically 3×10^{10} cells
and 1×10^7 CFU-GM for a typical 70 kg patient. When a starting
population of PBMC's from patients obtained in this fashion is
used for expansion in accordance with the invention, an 11 fold
increase in the CFU-GM population can be obtained in 14 days.

Thus, leukapheresis could be reduced 11 fold. Alternatively, a greater amount of GM progenitors can be transplanted. If 3.5 x 10^7 CFU-GM are desired from the expansion process, enriched PBMC's amounting to 3.2 x 10^6 CFU-GM would be required for the starting population. Since fewer enriched PBMC's would be required, two thirds of the current leukapheresis process could be avoided. Providing an increased amount of CFU-GM would benefit the patient by providing a greater number of the progenitors necessary to provide engraftment.

The cells may be expanded in accordance with the invention before or after freezing thereof. After chemotherapy, the expanded cells are reinfused into a patient by procedures known in the art.

The following examples are provided to further illustrate and describe the present invention; however, the scope of the present invention is not intended to be limited thereby.

Example 1

Fresh peripheral blood leukocytes were obtained by leukapheresis from a patient being treated with G-CSF therapy. The peripheral blood mononuclear cell (PBMC) fraction was obtained by density centrifugation of the heparinized blood cells by centrifugation (2000 rpm x 30 minutes) using Histopaque (Sigma, density=1.077). A portion of these cells were frozen in liquid nitrogen by the addition of 10% dimethyl sulfoxide and slow freezing. Fresh or previously frozen PBMC's were seeded into in vitro cultures using duplicate six well plates (volume = 3.0 mL). The seed density for both fresh and frozen cultures was 5.0 X 10^5 viable cells/mL. Tissue culture medium used was IMDM with 10% FBS, 100 units/mL penicillin, 100 mcg/mL streptomycin and cytokines. Added cytokines included IL-3 (0.5 ng/mL), GM-CSF (0.25 ng/mL), and c-kit ligand (also known as stem cell factor) (100 ng/mL) and were added at a rate of three times per week regardless of the medium feed schedule.

Medium was replaced as necessary in order to keep the cell concentration under 10^6 cells/mL. During periods of relatively constant cell number, medium was replaced on a weekly basis by removing one half of the volume of the culture, centrifuging and adding back the cells with fresh medium. During the period of rapid cell expansion, the cultures were split 1 to 2 in order to reduce the cell concentration. This was performed by removing and discarding half of the volume of the culture (along with cells) and replacing the volume with fresh medium. Cell counts were obtained every 2-3 days.

Total cell expansion data for six-well plates are presented in Figure 3. For both the fresh and frozen cultures, duplicates were in good agreement. Growth profiles exhibited a lag phase of 20 days before expanding. Following the period of rapid expansion, the fresh culture reached a total expansion of 16-18 - fold while the frozen culture expanded 28-35 fold.

Example 2

Fresh and frozen PBMC's obtained as described in Example 1 were seeded into 250 mL stirred tank bioreactors at a density of 5.0×10^5 viable cells/mL. Tissue culture medium and cytokine compositions and feed schedules were performed as described in Example 1.

Cell counts were performed at 2-3 day intervals. Starting at day zero and at weekly intervals thereafter, the spinner cultures were sampled for granulocyte-macrophage (CFU-GM) and erythrocyte (BFU-e) progenitor cells using methylcellulose colony assays (CytoMed). Cells were seeded into six well plates at densities ranging from 2.0×10^4 to 1.0×10^5 cells per plate using a volume of 1.0 ml. For each experiment, duplicate wells each at two different seeding densities were used to evaluate progenitor cell concentrations. Assay plates were scored for the presence of CFU-GM and BFU-e colonies at day 14. Colony assays

were performed on both spinner vessels as well as fresh and
frozen PBMC seed cultures.

The increase in total cell number was calculated using the
initial and final cell concentrations as well as the number of
splits using the formula:

Cell number = (Final cell conc./initial cell conc.) x 2^n

where n is the number of 1 to 2 splits. The increase in CFU-GM
and BFU-e was calculated using the increase in the total cell
number on the appropriate day as well as the concentration of
protenitor cells determined in the colony assays.

Total cell expansion data are presented in Figure 4 and
Table I. The spinner vessels exhibited a lag period of 10 and 20
days for the fresh and frozen cultures respectively. After the
period of expansion, the total increase for the fresh culture was
six-fold while the total cell expansion for the previously frozen
culture was nine-fold.

CFU-GM and BFU-e progenitor expansion data for spinner
vessels are presented in Figures 5 and 6 respectively. Maximal
expansion of GM progenitors was observed on day 21 for both
cultures. While the fresh culture expanded 12 fold, the frozen
culture exhibited an expansion of 42 fold. BFU-e progenitor
expansion was maximal on day 7. Here, the BFU-e content in the
fresh culture increased 110% while the frozen culture increased
40%.

Example 3

Bone marrow aspirates obtained from breast cancer patients
were centrigued (3000 rpm for 10 minutes) and nucleated cell
fractions (buffy coats) were removed and frozen for shipment.
Upon thawing, marrow was washed with IMDM with 10% FBS, 20 units/
ml heparin, and 500 units/ml DNase. The prepartion was enriched
for low-density mononuclear cells (LDMC) by density gradient
centrifugation. LDMC's were seeded into two 250 ml stirred tank

bioreactors at a concentration of 5.0 x 10^5 cells/mL using IMDM with 10% FBS and added cytokines. One vessel was operated as a suspension bioreactor while the second vessel was operated with 2.5 g/L of microcarrier beads for cell culture in order to support the growth of anchorage-dependent cells. The medium feeding schedule for both vessels was performed as described in Example 1. Added cytokines included IL-3 (0.5 ng/ml), GM-CSF (0.25 ng/mL), and kit ligand (stem cell factor) (100 ng/mL). Cytokines were added independent of the media changes at a rate of three times per week.

At weekly intervals, the cultures were sampled for progenitor assays. Granulocyte-macrophage (CFU-GM) and erythrocyte (BFU-e) progenitor assays were performed by inoculating cells into 35 mm culture dishes in duplicate as described in Exampe 2. Plates were scored for the presence of colonies at day 14. Assays were performed weekly in spinner vessels with and without microcarriers as well as freshly thawed bone marrow.

Results of the total cell increase for the vessels with and without microcarrier beads are presented in Table 1 and Figure 1. The vessels with and without microcarrier beads exhibited a maximum total cell expansion of 35 and 8 fold respectively. The maximum expansion of both CFU-GM and BFU-e progenitors were observed on day 7. The CFU-GM population increased from a normalized value of 1.0 to values of 2.5 and 1.8 for the suspension and microcarrier cultures respectively. The BFU-e population increased from 1.0 to 1.6 and 1.9 for the two cultures respectively.

Example 4

Bone marrow obtained and processed as described in Example 3 was inoculated into an airlift bioreactor at a concentration of 1.1 x 10^5 cells/mL using IMDM with 10% FBS with added cytokines at a volume of 575 mL. Medium and cytokine concentrations,

feeding rat s, and pr genitor assays were foll wed as described
in Example 3.

Results for the increase in total cells and progenitors are
presented in Table 1 and Figure 2. The total cell increase in
the airlift bioreactor was 5.3 fold on day 21. The CFU-GM
population expanded maximally on day 15 to a value of 2.1
compared to a normalized starting value of 1.0. No increase in
BFU-e progenitors were observed over what was initially seeded
into the culture.

Table I

Example	Vessel	Cell Source	Maximum increase in cell number[1]	Increase in CFU-GM[1]	Increase in BFU-e[1]
3	250 ml spinner vessel	Frozen Marrow	35 (day 36)	2.5 (day 7)	1.6 (day 7)
3	250 ml spinner vessel with microcarriers	Frozen Marrow	8 (day 32)	1.8 (day 7)	1.9 (day 7)
4	Airlift vessel	Frozen Marrow	5.3 (day 21)	2.1 (day 15)	no expansion
2	250 ml spinner vessel	Fresh PBMC's	5.5 (day 18)	14 (day 21)	2.1 (day 7)
2	250 ml spinner vessel	Frozen PBMC's	9 (day 28)	42 (day 21)	1.4 (day 7)

[1]The increase in total cell number was calculated using the initial and final cell concentrations as well as the number of splits using the formula:

Increase = (Final cell conc./initial cell conc.) x 2^n

where n is the number of 1 to 2 splits. The increase

in CFU-GM and BFU-e were calculated using the increase

in the total cell number on the appropriate day as well

as the concentration of progenitor cells determined in

the colony assays.

While the present invention has been described in
conjunction with specific embodiments thereof, it is evident that
many alternatives, modifications and variations will be apparent
to those skilled in the art in view of the foregoing description
and examples. Accordingly, modifications and variations in
following within the broadest scope and spirit of the following
claims.

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What is claimed is:

1.   A proc ss for _in vitro_ supp rt of mammalian hematopoietic cells derived from peripheral blood comprising:

maintaining hematopoietic cells derived from peripheral blood in a culture medium containing at least one cytokine effective for supporting said cells.

2.   A process as in Claim 1, wherein said hematopoietic cells are progenitor cells or stem cells.

3.   A process as in Claim 2, wherein said cells are maintained in a cell culture device.

4.   A process as in Claim 3, wherein said bioreactor is selected from the group consisting of: T-flasks, blood bags, perfusion chamber, stirred tank and airlift bioreactors.

5.   A process as in Claim 2, wherein said cytokine is at least one member selected from the group consisting of IL-1; IL-3; IL-6; GM-CSF; and c-kit ligand.

6.   A process as in Claim 2, wherein said cells are obtained from a patient's peripheral blood.

7.   A process as in Claim 6, wherein said patient was treated with cytokines to enrich progenitor cells prior to deriving said progenitor cells from the patient's peripheral blood.

8.   A process for expanding progenitor cells or stem clls derived from peripheral blood or bone marrow comprising:

expanding said cells in the presence of at least one cytokine in a cell culture device.

9.   A process as in Claim 8 wherein said device is a T-flask, blood bag, perfusion chamber, stirred tank bioreactor or airlift bioreactor.

10.   Hematopoietic cells expanded by the process of Claim 1.

11.   A process for treating a patient by cell replacement therapy comprising:
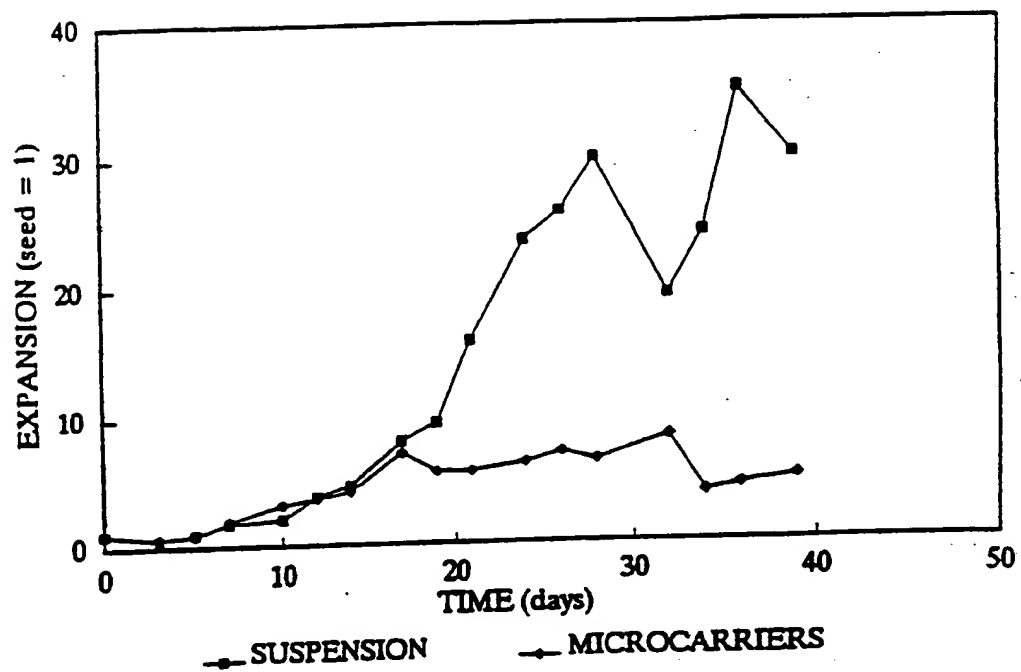
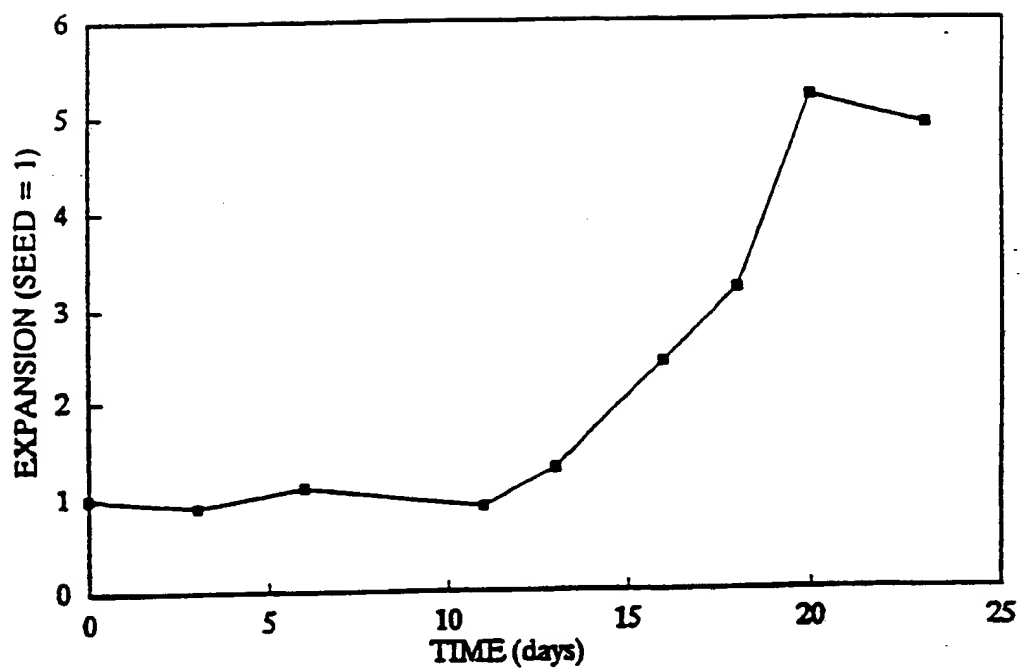administering to the patient the cells of Claim 10.
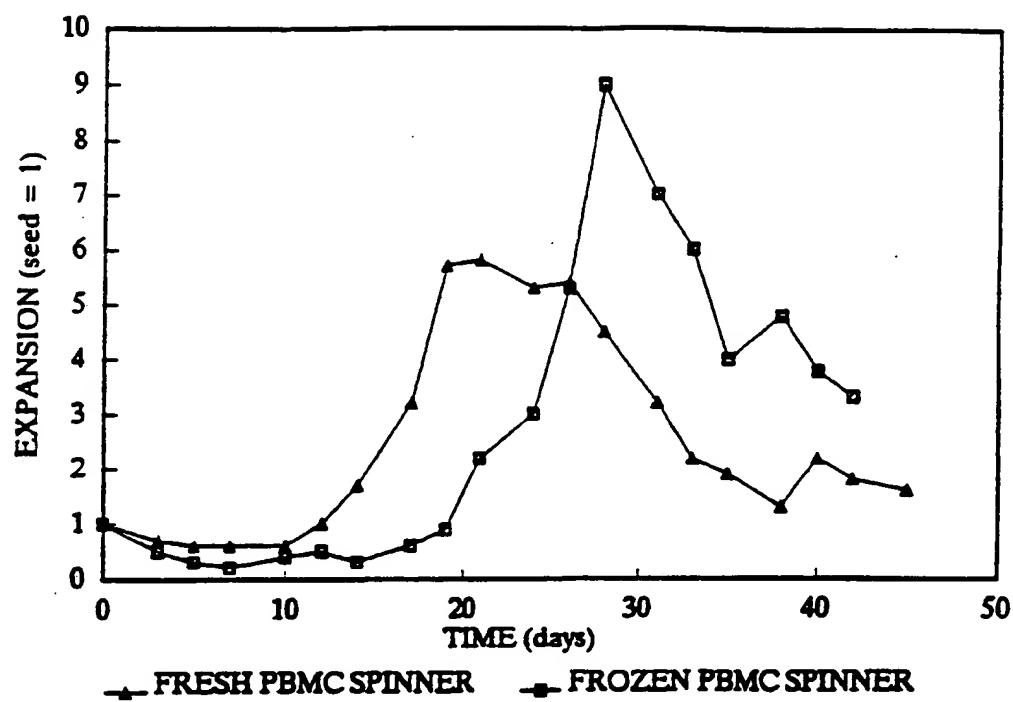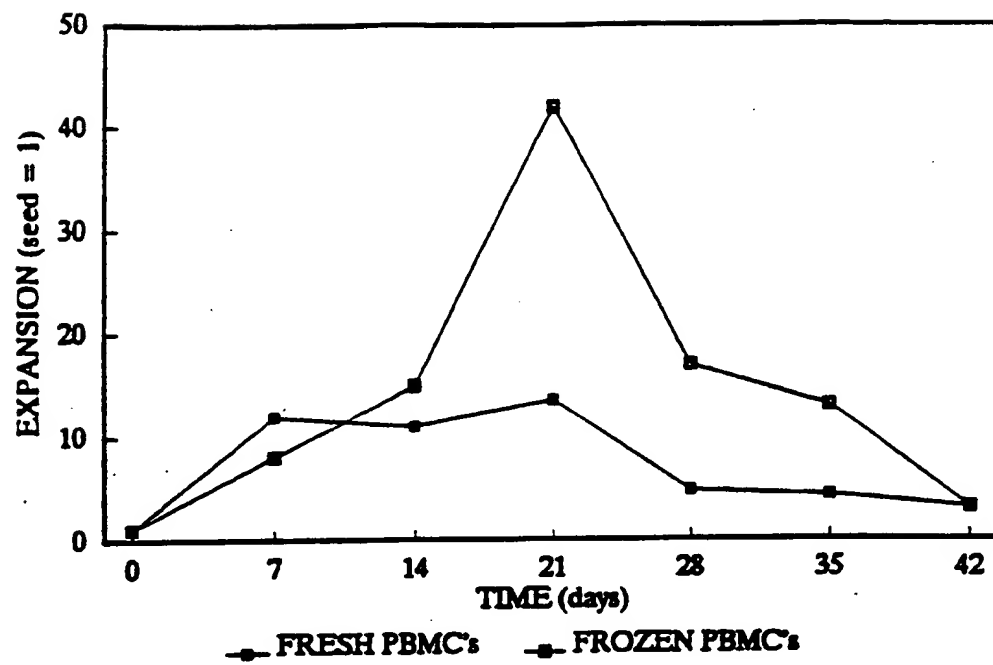
Figure 1

Figure   2

Figure 3

Figure   4

Figure 5

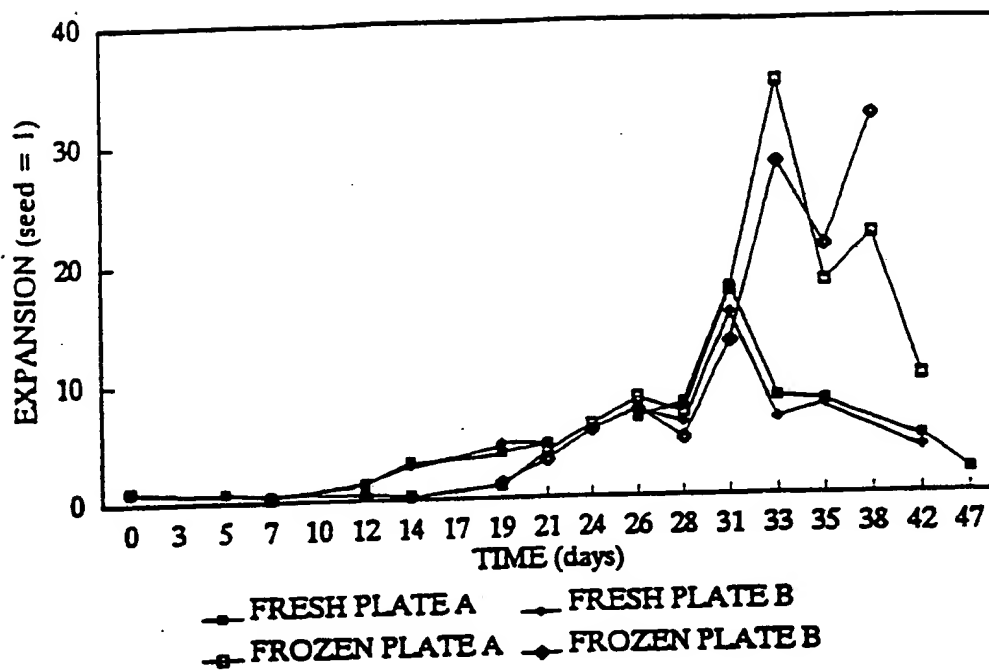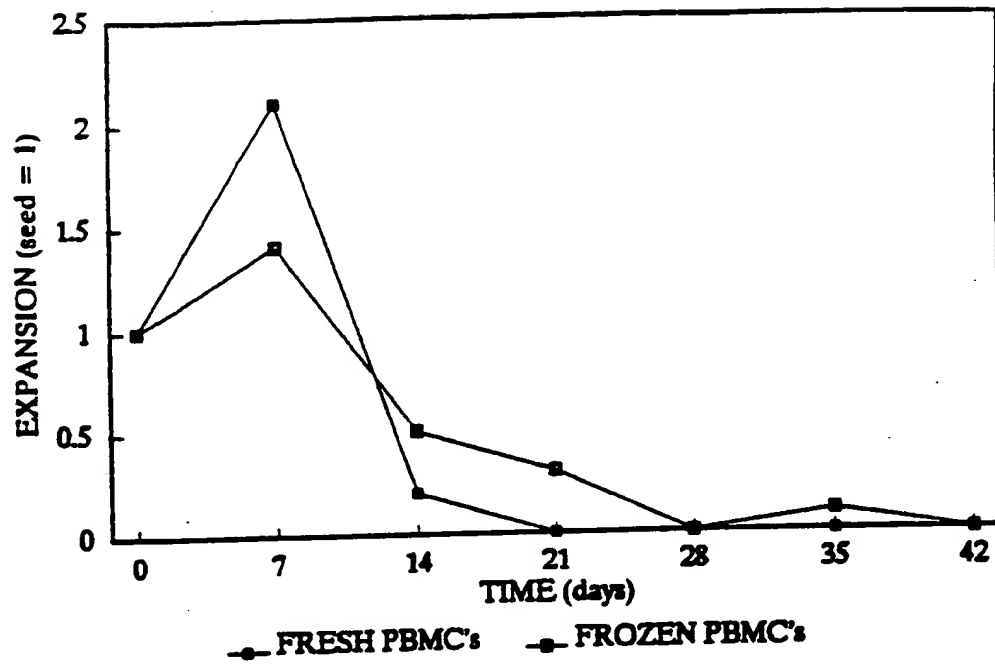Figure 6

INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

IPC(5) :C12N 5/00; A61K 37/00
US CL :435/240.2, 240.21, 240.3; 424/93U

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/240.2, 240.21, 240.3; 424/93U

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

BIOSIS
search Terms: hematopoietic, cytokine?, expan?

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US, A, 5,004,681 (Boyse et al) 02 April 1991, see entire document.	1-11
X,E	US, A, 5,192,553 (Boyse et al) 09 March 1993, see entire document.	1-11
A	US, A, 5,061,620 (Tsukamoto et al) 29 October 1991, see entire document.	1-11
A,P	US, A, 5,154,921 (Sager et al) 13 October 1992, see entire document.	1-11

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be part of particular relevance		
"E"	earlier document published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)		
		"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
02 JUNE 1993	14 JUN 1993

Name and mailing address of the ISA/US	Authorized officer
Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231	SUSAN M. WEBER
Facsimile No. NOT APPLICABLE	Telephone No. (703) 308-0196

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C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT		
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X,P	Biosis Abstract, Blood, Volume 81, No. 3, issued 1993, Srour et al, "Long-Term Generation and Expansion of Human Primitive Hematopoietic Progenitor Cells In-Vitro", pages 661-669, see abstract.	1-11
Y,P	Biosis Abstract, Biotechnology Progress, Volume 9, No. 2, issued 1993, Sardonini et al, "Expansion and Differentiation of Human Hematopoietic Cells from Static Cultures Through Small-Scale Bioreactors", pages 131-137, see abstract.	1-11
X,P	Biosis Abstract, Leukemia (Basingstoke), Volume 6, No. 10, issued 1992, Terstappen et al, "Differentiation and Maturation of Growth Factor Expanded Human Hematopoietic Progenitors Assessed by Multidimensional Flow Cytometry", pages 1001-1010, see abstract.	1-11
X,P	Biosis Abstract, Hokkaido Journal of Medical Science, Volume 67, No. 5, issued 1992, Han, M., "Synergistic Effects of Murine Stem Cell Factor in Combination with a Variety of Cytokines on the Expansion of Murine Hematopoietic Progenitor Cells in Short-Term Suspension Cultures", pages 674-683, see abstract.	1-11
X	Biosis Abstract, Acta Haematologica Japonica, Volume 53, No. 7, issued 1990, Okano et al, "A Novel Liquid Culture System for In-Vitro Expansion of Human and Murine Hemopoietic Stem Cells Using IL-6 and IL-3", pages 1213-1221, see abstract.	1-11
X	Biosis Abstract, Experimental Hematology (New York), Volume 20, No. 3, issued 1992, Muench et al, "Interactions among Colony-Stimulating Factors IL-1-Beta IL6 and Kit-Ligand in the Regulation of Primitive Murine Hematopoietic Cells", pages 339-349, see abstract.	1-11